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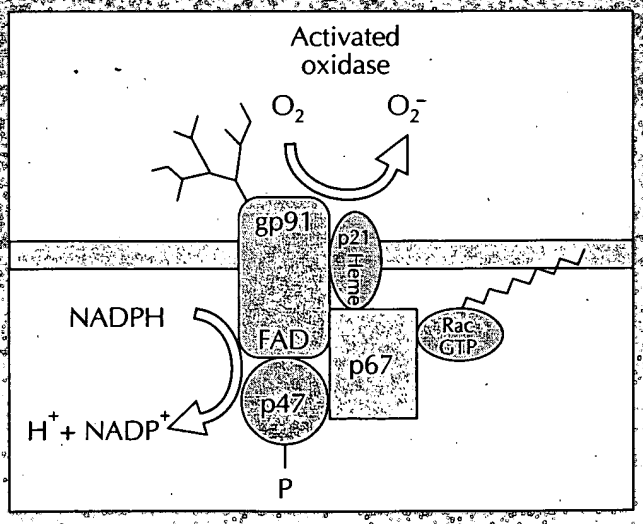

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Eosinophils: structure and functions

Peter F Weller

Harvard Medical School and Beth Israel Hospital, Boston, USA

Although much has been learned about the basic contents and capabilities of eosinophils, some of the roles eosinophils play in host defense and the immunopathogenesis of diseases remain enigmatic. In addition to containing four notable cationic granule proteins and their ability to synthesize lipid mediators of inflammation, eosinophils have recently been shown to be able to elaborate a range of cytokines that may exert autocrine as well as paracrine effects. The roles of eosinophils within tissues are modulated by interactions with the extracellular matrix and other cells during eosinophil recruitment and activation, and eosinophils may engage in cooperative interactions with other cells.

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Introduction

The eosinophil is a terminally differentiated, end-stage leukocyte that resides predominantly in submucosal tissues and is recruited to sites of specific immune reactions, including allergic diseases. The eosinophil has well established functional responses characteristic of end-stage effector leukocytes. Eosinophils can elaborate specific lipid mediators, including leukotriene C_4, platelet activating factor (PAF) and lipoxins, which are candidate mediators of acute allergic reactions (reviewed in [1]). In addition the eosinophil can release from its granules several distinctive cationic proteins that have the potential to cause local tissue damage and dysfunction, thereby contributing to the pathogenesis of local inflammation. Recent studies have also shown eosinophils to be sources of cytokines and to be capable of cooperative interactions with lymphocytes and other cells. This review will summarize recent advances in our understanding of the functions of eosinophils, including their capacity to form cytokines and the processes involved in their recruitment and activation.

Structure and contents

The morphological hallmark of the eosinophil is its content of specific cytoplasmic granules, in which the distinctive cationic proteins of the eosinophil reside. These specific granules are the principal identifying feature of eosinophils. The granules contain a unique crystalloid core, discernible at the ultrastructural level, composed of major basic protein (MBP); at the level of light microscopy this crystalloid core is responsible for the cardinal tinctorial properties of the eosinophil. Specific granules contain four distinct cationic proteins, which exert a range of biological effects on host cells and microbial targets [2]. Gleich and colleagues [3•] have quantitated the amount of each of these proteins in eosinophils, basophils and neutrophils. As measured by specific radioimmunoassays and expressed in ng (and nmol) per million cells they contained 8 982 (642) of MBP, 3 283 (178) of eosinophil-derived neurotoxin (EDN), 5 269 (251) of eosinophil cationic protein (ECP) and 12 174 (172) of eosinophil peroxidase (EPO). Basophils contained about one fourth as much MBP as did eosinophils, and contained detectable amounts of EDN, ECP and EPO, although at levels less than 7% of those in eosinophils. Small amounts of EDN and ECP were also present in neutrophils and may be synthesized by these cells [3•]. Thus, eosinophils are the dominant source of these four markedly cationic proteins. The properties of these proteins, and their numerous biological effects have been reviewed by Gleich [2] as these proteins have major effects not only on the potential role of eosinophils in host defense against helminthic parasites, but also in contributing to tissue dysfunction and damage in eosinophil-related allergic and other diseases. As MBP lacks enzymatic activity,

Abbreviations

ECP—eosinophil cationic protein; **EDN**—eosinophil-derived neurotoxin; **ELISA**—enzyme linked immunosorbent assay; **EPO**—eosinophil peroxidase; **GM-CSF**—granulocyte-macrophage colony-stimulating factor; **ICAM**—intercellular adhesion molecule; **IL**—interleukin; **MBP**— major basic protein; **MIP**—macrophage inflammatory protein; **PAF**—platelet activating factor; **TGF**—transforming growth factor; **TNF**—tumor necrosis factor; **VCAM**—vascular cell adhesion molecule; **VLA**—very late activation antigen.

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one mechanism whereby this highly cationic polypeptide may exert its activities is by interactions with lipid membranes. MBP associates with acidic lipids and disrupts, aggregates, fuses and lyses liposomes prepared from such lipids. Such interactions might contribute to MBP's wide range of toxicity [4]. In addition, both MBP and EPO, but not EDN or ECP, have been shown to act as selective allosteric inhibitors of agonist binding to M2 muscarinic receptors [5•]. Thus, these proteins may contribute to M2 receptor dysfunction and enhance vagally mediated bronchoconstriction in asthma.

There may be deficiencies in eosinophil granule contents. Isolated EPO deficiency occurs rarely and is associated with no clinical disorders [6]. It has been established that a syndrome characterized by defective specific granule formation in neutrophils also involves the eosinophil lineage. Eosinophils cannot be recognized morphologically by light microscopy as they lack formed specific granules, but may be identified by their content of EPO. In these EPO positive eosinophils, ECP, EDN and MBP proteins are absent, although mRNA transcripts for each can be detected [7].

The nature of proteases in human eosinophils curiously has been a little investigated topic. Using *in situ* hybridization to detect mRNA transcripts and immunohistochemistry, it has been demonstrated that eosinophils are a major source of a 92 kDa metalloproteinase, a gelatinase [8].

Cytokine production

Only recently has it been recognized that eosinophils are capable of elaborating cytokines, and the identities and activities of these cytokines are presently being investigated. While the possible activities of the eosinophil-derived cytokines are pleiotropic, these cytokines include those with potential autocrine growth-factor activities for eosinophils and those with potential roles in acute and chronic inflammatory responses.

Three cytokines have growth-factor activities for eosinophils, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3, and IL-5 [9•]. Eosinophils can synthesize each of these. Eosinophils contain mRNA transcripts for GM-CSF [10,11], IL-3 [10], and IL-5 [9•]. Evidence for active cytokine production by eosinophils includes the demonstration that eosinophil viability-sustaining activities of IL-3 and GM-CSF were present in supernatant fluids of ionomycin-stimulated eosinophils [10]. *In situ* hybridization studies have indicated that eosinophils participating in allergic and other responses in tissues may express these cytokines. Broide and colleagues [12••] have demonstrated that eosinophils recovered by bronchoalveolar lavage from asthmatics, before and after endobronchial allergen challenge, were induced to express GM-CSF and IL-5 mRNA, detected by *in situ* hybridization. Using dual labeling *in situ* hybridization, it was shown that expression of these two cytokines in eosinophils recovered after allergen challenge was not uniform: 34% of eosinophils were positive for both IL-5 and GM-CSF, 34% were positive for IL-5 but negative for GM-CSF, 11% were positive for GM-CSF and negative for IL-5 and 21% were negative for both cytokine transcripts. GM-CSF mRNA transcripts have been detected in eosinophils in nasal polyps [13], and IL-5 transcripts in intestinal tract eosinophils in patients with coeliac disease [9•]. Thus, eosinophils can elaborate three cytokines that promote the survival of eosinophils, antagonize apoptosis [14], and enhance effector responses of these cells. The differential regulation of cytokine production within eosinophils in tissue sites remains to be delineated. A role for adhesion proteins in mediating the enhanced production of GM-CSF in eosinophils has been indicated by the finding that eosinophils that adhere to fibronectin have enhanced survival *in vitro* and increased production of GM-CSF and IL-3 proteins [15], as well as enhanced effector responses. The increased eosinophil survival was inhibited by antibodies to either IL-3 or GM-CSF as well as by blocking antibodies to fibronectin and VLA-4, implicating VLA-4 expressed on eosinophils in transducing signals for heightened eosinophil production of IL-3 and GM-CSF.

Other cytokines elaborated by human eosinophils that may have activities in acute and chronic inflammatory responses include IL-1α [16••] (as also shown for murine eosinophils [17]), IL-6 [18,19], and IL-8 [20]. In addition, tumor necrosis factor (TNF)-α is elaborated by eosinophils [21••]. Using *in situ* hybridization, 44–100% of the blood eosinophils from five patients with hypereosinophilia and four normal subjects were found to exhibit intense hybridization signals for TNF-α mRNA. TNF-α protein was detectable by immunohistochemistry in blood eosinophils of hypereosinophilic subjects, and purified blood eosinophils from three atopic donors exhibited cycloheximide-inhibitable spontaneous release of TNF-α *in vitro* [21••]. At least some of the TNF-α in human eosinophils can be detected by immunogold ultrastructural localization within eosinophil-specific granules [22]. Within tissues, eosinophils infiltrating nasal polyps [21••] and those present in necrotizing enterocolitis [23] have been demonstrated to contain TNF-α mRNA by *in situ* hybridization. Eosinophils from eosinophilic donors and eosinophils infiltrating nasal polyps have also been shown by *in situ* hybridization to contain mRNA transcripts for macrophage inflammatory protein (MIP)-1α [21••].

Human eosinophils can elaborate both transforming growth factor (TGF)-α [24] and TGF-β [25]. The mRNAs for these cytokines, detected in tissue-dwelling eosinophils by *in situ* hybridization, have been demonstrated in eosinophils infiltrating nasal polyps [26,27]. While these cytokines may have roles in contributing to epithelial hyperplasia and fibrosis [28], it is likely that TGF-β also has regulatory effects on eosinophil survival and functioning as it has been shown to inhibit IL-3-dependent growth of eosinophils [29].

Recruitment of eosinophils

The processes that lead to the accumulation of eosinophils within tissue sites of specific inflammation, as for other leukocytes, involve numerous sequential interactions that enable eosinophils to adhere to and then transmigrate through the endothelium and to respond to local chemoattractants (reviewed in [30]). A preferential accumulation of eosinophils in tissue sites, as at allergic reactions, results from these serial interactions and is not dependent only on a chemoattractant highly specific for eosinophils. Indeed, chemoattractants that stimulate eosinophil migration, but also have other activities, continue to be identified, including the chemokine RANTES [31–34], cyclophilin [35] and the less potent MIP-1α [31].

The adhesion of eosinophils to endothelium potentially involves several pathways, including CD18-dependent pathways, adherence to E-selectin and P-selectin and adherence to VCAM by means of VLA-4 expressed on the eosinophil (reviewed in [30]). Initial binding to IL-1β stimulated endothelium involves VCAM, E-selectin and ICAM-1, whereas subsequent migration relies heavily on ICAM-1 and CD11/18 [36]. It is possible to block migration of eosinophils with an antibody to the α-chain of VLA-4 (α4). Dermal accumulation of guinea pig eosinophils into sites of passive cutaneous anaphylactic reactions or sites of inflammatory mediator release (e.g. PAF, C5a and leukotriene B_4) was inhibited by a monoclonal antibody to α4 given either intravenously or used to pretreat eosinophils [37]. The dynamic interactions that occur with eosinophil adhesion and migration are indicated by studies using an activating antibody to the β1 integrin chain. This antibody, which activates β1 integrins into a high-avidity binding state by 'freezing' β1 integrins in an activated state, inhibited eosinophil migration across extracellular matrix or endothelial cells [38]. Endothelial cells themselves may have a role in facilitating eosinophil transmigration [39], and migration of eosinophils across endothelium leads to enhanced CD11b and CD35 expression on eosinophils [40].

In experimental animals, allergen-induced recruitment of eosinophils into lung tissues may depend on $CD4^+$ T cells, presumably T helper type 2 cells, and the cytokines released by such T cells. In mice, interferon-γ inhibited antigen-induced eosinophil recruitment into airways by inhibiting $CD4^+$ T-cell infiltration [41]. In the guinea pig, allergen-induced airway eosinophilia and hyper-reactivity were largely mediated by IL-5 and were abrogated by anti-IL-5 administration [42]. In humans, IL-5 and to a lesser extent GM-CSF were the predominant eosinophil-active cytokines in the antigen-induced pulmonary late-phase reactions [43]. The accumulation of eosinophils in tissues, as in chronic asthma or acute antigen challenges in the lungs, correlates with measures of local T-cell activation. For instance, increases in activated T lymphocytes, eosinophils, and expression of cytokine mRNA for IL-5 and GM-CSF have been documented in bronchial biopsies after allergen inhalation challenge of atopic asthmatics [44].

Cytokine priming of eosinophils *in vivo*

It has long been recognized that eosinophils from eosinophilic donors exhibit metabolic, morphological, and functional changes that are indicative of their being 'activated' *in vivo*. Ongoing studies continue to provide evidence of this activation or priming, and to implicate the growth factor cytokines that act on eosinophils in this process. Evidence of this priming includes the demonstration that eosinophils obtained after allergen bronchoprovocation in asthmatics, in contrast with eosinophils from normal donors, exhibited enhanced chemotactic responses to formyl-peptide and IL-8 [45]. Similar findings of *in vivo* priming of chemotactic responses have been found with eosinophils from donors with atopic dermatitis [46]; the primed responses can be elicited *in vitro* by the exposure of eosinophils to IL-5 or GM-CSF [46–48]. The mechanisms for this cytokine priming of eosinophils have not been fully defined, but increased protein kinase C activity has been demonstrated in such eosinophils [49]. Cytokine priming of the eosinophil respiratory burst has been associated with tyrosine-kinase activation [50].

Functions of eosinophils

The eosinophil as an effector cell may elaborate lipid mediators, including leukotriene C_4, lipoxins and PAF (reviewed in [1]), and release four distinctive cationic granular proteins that may contribute to the acute and later manifestations of allergic or other immunological responses. A diversity of past and recent [51,52] epidemiological and clinical investigations correlate eosinophil involvement with the pathogenesis of the manifestations of allergic diseases. Thus, eosinophils clearly have a functional role in allergic inflammation.

The more obviously beneficial function of the eosinophil has been related to its role in host defense against helminthic parasite infections. The results of recent studies, however, in which the administration of neutralizing antibody to IL-5 has been used to abrogate the development of eosinophilia in helminth-infected mice, have caused this role to be re-evaluated. In mice experimentally infected with different helminthic parasites, including *Trichinella* [53•], anti-IL-5 antibody has prevented the post-infection blood, marrow and tissue eosinophilia. In these eosinophil-deficient mice treated with anti-IL-5 (or anti-IL-4) antibody, however, the intensities of neither primary nor secondary infections were greater than in eosinophilic mice [54,55]. Helminthic infections in other host species have not yet been evaluated, so whether these findings, arguing against a major role of eosinophils as helminthotoxic effector cells, are limited to mice remain to be defined. While it is possible that other mechanisms of host defense against helminths may be sufficiently redundant that eosinophil ablation is not deleterious, these findings with anti-IL-5 depletion of eosinophils

raise the possibility that eosinophils do not have a predominant function in parasite host defense.

Other potential functions for the eosinophil have not yet been fully defined. In addition to the acute release of lipid, peptide and cytokine mediators of inflammation, eosinophils likely contribute to chronic inflammation, including the development of fibrosis. Eosinophils are the major source of the fibrosis-promoting cytokine TGF-β in nodular sclerosing Hodgkin's disease [28]. Additional roles for the eosinophil in modulating extracellular matrix deposition and remodeling are suggested by studies of normal wound healing. During dermal wound healing eosinophils infiltrate into the wound sites and sequentially express TGF-α early, and TGF-β later, during wound healing [56].

Additional functions for eosinophils are suggested by the finding that eosinophils may be induced to express MHC class II proteins and can function as antigen-presenting cells [16••,57,58]. Blood eosinophils lack HLA-DR expression but eosinophils recovered from the airways 48 hours after segmental antigen challenge have been shown to express HLA-DR [59•]. Cytokines, including GM-CSF, IL-3, IL-4 and interferon-γ, induce eosinophil HLA-DR expression [16••]. The cytokines IL-3, interferon-γ, TNF-α and TNF-β can induce blood eosinophils to express ICAM-1, which facilitates adhesion to autologous T cells [57,60]. Both murine and human eosinophils can function as HLA-DR dependent, MHC-restricted antigen-presenting cells in stimulating proliferation of human T cells or T-cell clones [16••,57,58].

Conclusion

The eosinophils have well delineated mechanisms for contributing to the acute pathogenesis of allergic inflammation. Their recruitment and activation are dependent on the activities of other cells including T lymphocytes and the cytokines that they secrete. The functions of eosinophils in other responses involving interactions with cells ranging from lymphocytes to fibroblasts remain to be more fully investigated.

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PF Weller, Department of Medicine, Harvard Medical School, and Beth Israel Hospital, 330 Brookline Avenue, Boston, Massachusetts 02215, USA.